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Research Paper

Anti-Microbial Activity of Essential Oil Extracted from Shea Tree Seed (*Butyrospermum parkii*) in Mubi, North-Easthern Nigeria

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Abstract: *Anti-microbial activity of essential oil extracted from Butyrospermum parkii seed against Candida albicans, Escherichia coli, Staphylococcus aureus, Streptococcus species, and Trichophyton species was conducted in Mubi, North-Eastern Nigeria. Antimicrobial screening protocol was carried out by agar dilution method. The result showed that the bio-efficacy of the oil is close to that of the standard antibiotic drugs when compared; the oil inhibited the growth of C. albicans and Trichophyton spp close to that of the standard drug (Co-trimoxazole), while E. coli, S. aureus and Streptococcus spp showed resistivity activity. The oil is hereby recommended for its antimicrobial property for therapeutic purposes and can be used as an alternative medicine against C. albicans, E. coli, S aureus, Streptococcus spp and Trichophyton spp.*

Keywords: Antimicrobial, Antifungal, bacteriostatic, and *Butyrospermum parkii*.

Introduction

Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties.[1] Essential oils are rich source of biologically active compounds. They are aromatic oily liquids, which are obtained from various plant parts such as flowers, buds, seeds, leaves, twigs, bark, woods, fruits and roots by steam distillation.[2] Most essential oils are clear and contain the true essence of the plant it was derived from.[3] Essential oils are complex mixtures comprising many

single compounds and each of these constituents contribute to the beneficial or adverse effects of these oils.[4]

Plant essential oils are rich source of scents and are used in food preservation and aromatherapy. They possess multiple antimicrobial i.e., antibacterial,[2] antifungal,[5] anticancer, antiviral and antioxidant properties,[6,7] against viruses, bacteria and fungi.[8] They also have good fighting potentials against drug resistant pathogens.[9,10]

Over the years, plant oils and extracts have been used for a wide variety of purposes.[11] These purposes vary from the use of rosewood and cedar wood in perfumery, to flavoring drinks with lime, fennel or juniper berry oil, and the application of lemongrass oil for the preservation of stored food crops.[12] In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies.[13,14]

The essential oils and their constituents have been found effective as antifungal agent. The oil extract of *Nigella sativa* showed *Invitro* and *Invivo* antimicrobial effect against *Staphylococcus aureus, Pseudomonas aeroginosa* and *Candida albicans*.[15] The inhibitory effects of aqueous extract of seed of *Nigella sativa* against *C. albicans* have also been shown *Invivo*.[16] Several reports have been made on the fungicidal properties of neem oil; [17,18] mustard seed oil.[19]

Although, essential oils extracted from plants have been proven highly potent antimicrobial agent, it is also necessary to further investigate the antimicrobial activity of shear tree seed especially on *Candida albicans, Escherichia coli, Staphylococcus aureus, Streptococcus* species and *Trichophyton* species.

Hence, this study tends to investigate the anti-microbial activity of Shea butter seed *Butryospermum parkii* on *Candida albicans, Escherichia coli, Staphylococcus aureus, Streptococcus* species, and *Trichophyton* species.

Materials and Methods

Extraction of Essential Oil from Shea Kernel

The oil was locally extracted by boiling the seed with its shell. It was allowed to dry for some days, and then the shells were broken and allowed to dry for about three days and then fried and allowed to cool. It was pounded using a mortar and pistil. Water was poured in a pot and set on fire. It was allowed to boil for a while, before the ground kernel was poured into the boiling water. Thereafter, using a stirrer, it was stirred thoroughly. After some time the intensity of the fire source was reduced under the pot, and a sample of potassium was added to the mixture in the pot. Immediately after adding the potassium sample, the mixture fumed. The stirring continued until it stopped fuming and the mixture settled down indicating that the oil is ready. The oil was removed and put into a basin and stored in a cool dry place. After 3-4 days, the oil was removed from the basin and cooked again until it became white in color, then it was put in an iron basin and ready for use.

Antimicrobial Assay

The antimicrobial screening protocol was carried out by agar dilution method as described by Mitscher *et al.*, [20] for the antimicrobial activity evaluation in higher plants' extracts. Extracted *B. parkii* oil was evaluated at a concentration of 1000 µl/ml in dimethyl sulfoxide (DMSO) by diluting with 10 ml of molten blood agar at 45-50°C. The agar and extracted oil were mixed thoroughly and the mixture were poured into a Petri dish on a leveled surface to obtain an even agar depth of about 3-4 mm. Inoculates for the screening assay were prepared by growing overnight cultures of bacteria in nutrient broth. The cultures were diluted to 1:1000 in broth to give 104 Colony Forming Units (CFU)

per µl of the inoculums. Plates for the determination of anticandidal activity of oil were prepared by dispensing 15 ml of sterile Sabouraud's Dextrose Agar (SDA) into 100 x 15 mm sterile Petri dishes. The inoculate were prepared by adding 1ml of overnight *Candida* cultures to 9 ml of nutrient broth to yield 104 colony forming units (CFU/ml) of the inoculums. Sterile cotton-tipped applicators were used to streak the entire surface of agar plates. Cylindrical plugs were removed from the solidified agar plates using sterile cork borer to produce wells having a diameter of approximately 11 mm. Then 100 µl of the volatile oil and/or extract were added to each well. Dimethyl sulfoxides (DMSO) were used as a negative control in all the experiments. The plates were incubated between 30-32°C for about 24 hours. Antimicrobial activities were recorded as the width (in millimeters) of the clear zone of inhibition surrounding the agar well. The results were reported as positive (+) if there was inhibition of growth and negative (-) if there was no inhibition of growth. Triplicate sets of plates were prepared on each occasion and experiments were repeated three times. The mean of three readings were calculated and used in the analysis. Minimum Inhibitory Concentrations (MICs) was determined after 24hrs for the bacteria and after 48hrs for *C. albicans*. The MICs was determined as the lowest concentration of oil inhibiting the visible growth of each organism on the agar plate.

Methods of Media Preparation

Preparation of Agar Plates

Nutrient Agar: 1.5g of agar powder was dissolved in 100ml of nutrient broth. It was sterilized at 121^0C for 5 minutes and was allowed to cool at 50^0C. The media was dispensed into sterile Petri dishes under aseptic condition and was allowed to solidify.

Blood Agar: Blood agar was prepared by dissolving 19.5g of Colombia blood agar base (oxide) in 475ml of distilled water and was then sterilized at 121^0C for 15 minutes. The media was allowed to cool to 50^0C. 35ml of detrinated horse blood was added and mixed thoroughly and dispensed into Petri dishes and allowed to solidify.

Sabouraud Dextrose Agar: Sabouraud dextrose agar was prepared by dissolving 65g in 1litre of distilled water in a conical flask; the content of the conical flask was autoclaved for homogeneity (sabouraud media instruction). When cooled, chlorophenicolacidon was added to the autoclaved content to inhibit bacterial and sporadic fungal contamination.[21] The media was dispensed into sterile 9cm Petri dishes under aseptic condition and was left to solidify.

Results

The oil showed resistivity effect on *Escherichia coli* at 100% dilution. The zone of inhibition was 2mm while the drug Ciprofloxin inhibited 6mm. At 25%, the oil showed no zone of inhibition while the drug Peflocin inhibited 4mm as seen in Table 1.

Table 1: Standard antibiotics disc and plant extract treated with *Escherichia coli*

Microorganism	Drugs	Zone of Inhibition of drugs in (mm)	Dilution of plant extract in (%)	Zone of inhibition of plant extract in (mm)	Conclusion
Escherichia coli	Ciprofloxin	6	100	2	Resistant
	Gentamycin	5	50	1	Resistant
	Peflocin	4	25	0	Resistant

Table 2 shows the resistivity effect of oil on *Staphylococcus aureus;* at 100% dilution.

Table 2: Standard antibiotics disc and plant extract treated with *Staphylococcus aureus*

Microorganism	Drugs	Zone of Inhibition of drugs in (mm)	Dilution of plant extract in (%)	Zone of inhibition of plant extract in (mm)	Conclusion
Staphylococcus aureus	Ciprofloxin	8	100	3	Resistant
	Gentamycin	4	50	1.5	Resistant
	Peflocin	5	25	0	Resistant

The zone of inhibition was 3mm while the drug Ciprofloxin inhibited at 8mm and at 25% the oil showed no zone of inhibition while the drug Peflocin inhibited 5mm as seen above.

Table 3 shows the resistivity effect of oil on *Streptococcus species.*

Table 3: Standard antibiotics disc and plant extract treated with *Streptococcus* species

Microorganism	Drugs	Zone of Inhibition of drugs in (mm)	Dilution of plant extract in (%)	Zone of inhibition of plant extract in (mm)	Conclusion
Streptococcus species	Ciprofloxin	4	100	4	Resistant
	Gentamycin	6	50	1.5	Resistant
	Peflocin	3	25	0	Resistant

At 100%, the zone of inhibition was 4mm. This coincides with that of the Ciprofloxin drug; and at 25% the oil showed no zone of inhibition while the drug Peflocin inhibited at 3mm.

In Table 4, the oil showed a highly sensitive effect on *Candida albican;* at 100% the zone of inhibition was 8mm which was more than that of the drug Co-trimoxazole, which inhibited at 5mm. And at 25%, the zone of inhibition was 1.2mm while the drug Flucamed inhibited at 7mm.

Table 4: Standard antibiotics disc and plant extract treated with *Candida albicans*

Microorganism	Drugs	Zone of Inhibition of drugs in (mm)	Dilution of plant extract in (%)	Zone of inhibition of plant extract in (mm)	Conclusion
Candida albicans	Co-trimoxazole	5	100	8	Highly sensitive
	Penicillin	6	50	4	Resistant
	Flucamed	7	25	1.2	Resistant

And in Table 5, the oil was effective on *Trichophyton* species.

Table 5: Standard antibiotics disc and plant extract treated with *Trichophyton* species

Microorganism	Drugs	Zone of Inhibition of drugs in (mm)	Dilution of plant extract in (%)	Zone of inhibition of plant extract in (mm)	Conclusion
Trichophyton species	Co-trimoxazole	4	100	8.5	Highly sensitive
	Penicillin	6	50	6	Moderately active
	Flucamed	7	25	1.5	Resistant

At 100% the zone of inhibition was 8.5mm which was more than that of the drug Co-trimoxazole which inhibited at 4mm and at 25% the zone of inhibition was 1.5mm while the drug Flucamed inhibited at 7mm as seen in Table 5 above.

Discussion

Shea Butter Activity against *Escherichia Coli*

In Table 1, essential oil of Shea butter was found to have bacteriocidal activity against *E. coli*. The zone diameters measured to the nearest mm were less significant compared to the standard zone diameter of ciprofloxin, gentamycin and peflocine disc respectively. The result of this study showed that *B. parkii* oil was bacteriostatic than the standard disc of the drug. This coincides with the work of Dorman and Deans, [22] who reported antimicrobial activity of rosemary and eucalyptus essential oils against *E. coli.* Ekpa and Ebana,[23] also reported that palm kernel oil "Mmanyanga" and two other oils:- palm oil and coconut oil, on some microorganisms revealed that "Mmanyanga" was active against *E. coli.*

Shea Butter Activity against *Staphylococcus Aureus*

The oil of Shea butter showed antimicrobial activity against *S. aureus* as seen in (Table 2). The zone of inhibition of the ciprofloxin, gentamycin and peflocine standard control disc of the drugs was more than that of the oil comparatively. Other works on different oils are in line with that of the Shea butter because of their antimicrobial properties, this is also significant because it shows bacteriostatic properties. The oil extract of *Nigella sativa* reported by Mashhadian and Rakhandeh,[15] showed *Invitro* antimicrobial effect against *S. aureus*. Brady *et al.*,[24] also reported some plant essential oils of Citrus (*Citrus lemon*), Olive (*Olea europaea*), Ajwain (*Trachiyspirum ammi*), Almond (*Amygdalus communis*), Bavchi (*Psoralea corylifolia*) and Neem (*Azadirachta indica*) have shown growth inhibitory effects against *S. aureus.*

Shea Butter Activity against *Streptococcus* Species

Shea butter showed inhibitory activity against *Streptococcus* species as seen in (Table 3). The zone of inhibition of the oil at 100% concentration was found to be the same with the drug ciprofloxin while the zone of inhibition of the oil at 50% and 25% were less than the standard drugs gentamycin and peflocine; therefore it is still resistance towards the bacteria.

Shea Butter Activity against *Candida Albicans*

The essential oils and their constituents have been found to be effective as antifungal agent against *C. albicans* as seen in (Table 4). The zone of inhibition at 100% was highly sensitive in inhibition on the fungi more than the standard drugs co-trimoxazole, while at 50% and 25% the zone of inhibition of the standard drugs penicillin and flucamed were more than that of the oil. Khan *et al*., [16] reported that the inhibitory effects of aqueous extract of the seed of *Nigella sativa* against *C. albicans* have also been shown *invitro.* Several reports have been made on the fungicidal properties of neem oil.[17,18] It was observed that mustard seed oil also showed antifungal activity as reported by Nielsen and Rios.[19] Cushnie *et al.*,[25] reported that chloroform oil extract exhibited the highest activity against the fungus *C. albicans.*

Shea Butter Activity against *Trichophyton* Species

Shea butter oil was found to possess antifungal activity against *Trichophyton* species as seen in (Table 5). The zone of inhibition at 100% concentration of oil was found to be highly sensitive compared with the standard disc of co-trimoxazole while at 50% concentration of the oil and the standard disc of

penicillin were found to have the same zone of inhibition which were moderately active but at the lowest concentration (25%). The inhibitory zone of the oil was less than that of the standard disc of flucamed. Nevertheless it showed resistivity activity on the fungus *Trichophyton* species.

Conclusion

From the study, it has been observed that the essential oil of Shea butter possess both bacterio-static and bactericidal activity much higher than that of synthetic antibiotics when tested *invitro*, to further confirm the effectiveness of essential oils.

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